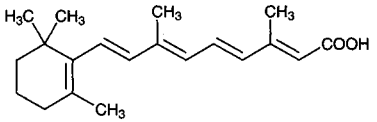
CHROMATOGRAM
Retention time: k' 0.38

OTHER SUBSTANCES
Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS
effect of mobile phase pH on capacity factor is discussed

REFERENCE
Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31–40.

Retinoic acid



Molecular formula: $C_{20}H_{28}O_2$
Molecular weight: 300.44
CAS Registry No.: 302-79-4 (tretinoin (all-trans)), 4759-48-2 (isotretinoin (13-cis)), 5300-03-8 (alitretinoin (9-cis))
Merck Index: 8333
Lednicer No.: 3 12 (isotretinoin)

SAMPLE
Matrix: blood
Sample preparation: Evaporate 25-100 μL IS in MeOH to near dryness under a stream of nitrogen. Add 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM KH_2PO_4 containing 40 mM Na_2HPO_4, pH 7.)

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
Flow rate: 1
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 24.9 (isotretinoin), 26.7 (tretinoin), 26.2 (alitretinoin (9-cis))
Internal standard: acitretin (21), 13-cis-acitretin (19.8)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

KEY WORDS
plasma; rabbit; rat

REFERENCE
Disdier,B.; Bun,H.; Catalin,J.; Durand,A. Simultaneous determination of all-trans-, 13-cis, 9-cis-retinoic acid and their 4-oxometabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 143–154.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 1.5 mL EtOH containing IS, freeze at -20° for 30 min, centrifuge. Inject a 1.4 mL aliquot of the supernatant onto column A and elute to waste with mobile phase A (time not given). Elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A C18-Corasil or C18-Lichrospher; B two 250 × 4 Supersphere 100 RP-18 endcapped columns in series
Mobile phase: A. MeCN:acetic acid (ratio not given) containing 1% ammonium acetate; B. Gradient. MeCN:acetic acid:10% ammonium acetate:water 60:1:6:30, 95:2:0.5:2, and 99:0.5:0:0.5 (times not given).
Detector: UV 360

CHROMATOGRAM
Internal standard: acitretin
Limit of quantitation: 300 pg/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-oxo-isotretinoin, 4-oxo-tretinoin

KEY WORDS
plasma; column-switching; pharmacokinetics; for isotretinoin and tretinoin

REFERENCE
Chen,C.; Mistry,G.; Jensen,B.; Heizmann,P.; Timm,U.; van Brummelen,P.; Rakhit,A.K. Pharmacokinetics of retinoids in women after meal consumption or vitamin A supplementation, *J.Clin.Pharmacol.*, **1996**, *36*, 799–808.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL serum with 200 μL MeCN and 10 μL 20 mM ascorbic acid, centrifuge at 16000 g for 5 min. Mix the supernatant with 200 μL water, inject a 2 μL aliquot. (Protect all solutions from light.)

HPLC VARIABLES
Column: 50 × 0.18 3 μm ODS-AQ (YMC, Wilmington, NC) (The separation capillary column was formed from fused-silica capillaries (Polymicro Technologies, Phoenix) by inserting a small 50 μm I.D. capillary ca. 15 mm into a larger 180 μm I.D. capillary and fixed by applying epoxy (No. 353ND, Epoxy Technology, Billerica MA). A glass filter paper frit (Whatman GF/A) was inserted into the larger capillary and forced against the smaller capillary with a stream of isopropanol. The stationary phase was suspended in 3 mL isopropanol and pumped into the larger capillary until a 50 mm bed was formed. The larger and smaller diameter capillaries extended no more than 100 and 16 mm from the frit, respectively.)
Mobile phase: MeCN:MeOH:water 65:2.5:32.5 containing 1% tetrabutylammonium perchlorate, adjusted to pH 5.0 with acetic acid and 174 mM sodium acetate
Flow rate: 0.004
Injection volume: 2
Detector: E, carbon-fiber working electrode +900 mV, Ag/AgCl reference electrode (details of preparation in paper)

CHROMATOGRAM
Retention time: 9.0 (isotretinoin), 9.5 (tretinoin)
Limit of detection: 410 pg/mL (isotretinoin), 64 pg/mL (tretinoin)
Limit of quantitation: 49.6 fmol (isotretinoin), 87.6 fmol (tretinoin)

OTHER SUBSTANCES
Extracted: retinaldehyde, retinol

KEY WORDS
cow; serum; capillary HPLC

REFERENCE
Hagen,J.J.; Washco,K.A.; Monnig,C.A. Determination of retinoids by reversed-phase capillary liquid chromatography with amperometric electrochemical detection, *J.Chromatogr.B*, **1996**, *677*, 225–231.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 750 μL 100 ng/mL acitretin in MeCN:9 mM NaOH 20:80, centrifuge at 1500 g for 3 min, inject a 500 μL aliquot onto column A with mobile phase A and elute for 7 min, elute column A in backflush mode with mobile phase A for 3 min, backflush contents of column A onto column B with mobile phase B and start the gradient for mobile phase B. At the end of the process flush the lines with component B of mobile phase B, re-equilibrate columns for 4 min. (Keep sample at 10° in the autosampler.)

HPLC VARIABLES
Column: A 14 × 4.6 37-50 μm Bondapak C18 Corasil (column fitted with 3 μm sieves not glass fiber filters); B 30 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1
Mobile phase: A MeCN:1% ammonium acetate 10:90; B Gradient. A was MeCN:water:10% ammonium acetate:acetic acid 600:400:4:30. B was MeCN:water:10% ammonium acetate:acetic acid 850:146:4:10. A:B 100:0 to 70:30 over 6 min, then to 0:100 over 5 min, stay at 0:100 for 11 min.
Flow rate: A 1.5; B 1
Injection volume: 500
Detector: UV 360

CHROMATOGRAM
Retention time: 25 (isotretinoin), 27 (tretinoin)
Internal standard: acitretin (23)
Limit of detection: 0.5-1 ng/mL
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Simultaneous: tretinoin, 4-oxoisotretinoin, 4-oxotretinoin, metabolites

KEY WORDS
plasma; column-switching

REFERENCE
Wyss,R. Determination of retinoids in plasma by high-performance liquid chromatography and automated column switching, *Methods Enzymol.*, **1990**, *189*, 146–155.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 9.44 (tretinoin)

OTHER SUBSTANCES
Extracted: isotretin, motretinid, acitretin, Vitamin A (retinol), retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1.25 g 0.1% isotretinoin cream or 0.05% isotretinoin gel, make up to 10 mL with MeOH, vortex for 15 min. Cut open two 10 mg isotretinoin capsules, add about 40 mL MeOH, sonicate for 10 min, filter, make up to 100 mL with MeOH. Grind 10% isotretinoin beadlets to powder, weigh out a 1.25 mg aliquot. Add 100 μL cream, gel, or capsule solution to 0.5 mL stainless steel extraction cartridge partially filled with Celite, complete filling with Celite, load the cartridge into the extraction chamber. Alternatively, directly add 1.25 mg beadlets powder to the extraction chamber (Supercritical Fluid Extractor, Isco, model SFX 2-10). Extract using the following conditions: chamber and restrictor temperature 45°, pressure 325 atm, static extraction time 2.5 min, dynamic extraction time 5 min, mobile phase MeOH:CO_2 5:95, solvent trap 17 mL MeOH. After extraction cool the MeOH extract to room temperature for 10 min, make up to 25 mL with MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeCN:MeOH:0.05% glacial acid 42.5:32.5:25
Flow rate: 1.2
Injection volume: 20
Detector: UV 360

CHROMATOGRAM
Retention time: 25.0 (isotretinoin), 30.3 (alitretinoin (9-cis)), 33.2 (tretinoin)

OTHER SUBSTANCES
Extracted: 11,13-di-*cis* retinoic acid, 9,13-di-*cis* retinoic acid, 9-*cis* retinoic acid, tretinoin

KEY WORDS
beadlets; capsule; cream; gel; SFE

REFERENCE
Simmons,B.R.; Chukwumerije,O.; Stewart,J.T. Supercritical fluid extraction of 13-cis retinoic acid and its photoisomers from selected pharmaceutical dosage forms, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 395–403.

SAMPLE
Matrix: milk
Sample preparation: Mix 50 mL milk with 30 mL ethanolic KOH (10:30). Saponify the mixture at 80° for 20 min. Extract twice with 10 mL n-hexane. Evaporate the extracts to dryness, reconstitute the residue with 1 mL mobile phase, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5μm C18 (Alltech)
Mobile phase: MeOH:EtOH 80:20
Flow rate: 1
Injection volume: 5
Detector: UV 250

CHROMATOGRAM
Retention time: 2.97 (isotretinoin), 3.38 (tretinoin)

OTHER SUBSTANCES
Extracted: retinal, vitamin A, vitamin D2, vitamin D3, vitamin E, vitamin K1, vitamin K2

REFERENCE
Gong,B.Y.; Ho,J.W. Simultaneous separation and detection of ten common fat-soluble vitamins in milk, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2389–2397.

SAMPLE
Matrix: silicone oils
Sample preparation: Condition a 1 g Si Bond-Elut SPE cartridge with 5 mL n-hexane. Mix 1 g silicone oil with 2 mL dichloromethane, vortex for 2 min, centrifuge at 3000 g. Withdrawn the supernatant, repeat this procedure twice, filter (0.45 μm), heat the filtrate at 50°, expose to a stream of helium for 30 min. Add 2.5 μg retinol acetate, 2.5 μg α-tocopherol acetate, and 25 μg BHT. Add the mixture to the SPE cartridge, elute with 500 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. A was MeCN:200 mM ammonium acetate 72:25. B was MeOH:water 95:5. A:B 100:0 for 10 min, to 0:100 over 1 min, maintain at 0:100 for 14 min
Flow rate: 2 for 10 min then 1.5
Injection volume: 20
Detector: UV 350

CHROMATOGRAM
Retention time: 2
Internal standard: retinol acetate (9.5)
Limit of detection: 79.2 ng/mL
Limit of quantitation: 264.1 ng/mL

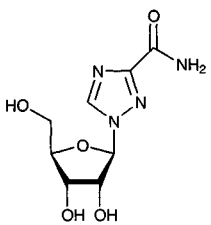
OTHER SUBSTANCES
Extracted: cholesterol (UV 210), retinal (UV 350), α-tocopherol acetate (UV 210), vitamin A (UV 350), vitamin E (UV 210)

KEY WORDS
ophthalmic silicone oils; SPE

REFERENCE
Del Nozal,M.J.; Bernal,J.L.; Marinero,P. Simultaneous HPLC determination of cholesterol, α-tocopherol, retinol, retinal and retinoic acid in silicone oils used as vitreous substitutes in eye surgery, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1151–1167.

Ribavirin

Molecular formula: $C_8H_{12}N_4O_5$
Molecular weight: 244.21
CAS Registry No.: 36791-04-5
Merck Index: 8365



SAMPLE
Matrix: blood, CSF
Sample preparation: Prepare a boronate affinity gel SPE column by packing 100 mg Affi-Gel 601 (Bio-Rad) into a 65 × 6 1.5 mL polypropylene column, condition with 10 mL buffer, store at 4°, immediately before use condition further with two 1 mL aliquots of buffer. 500 μL Serum, plasma, or CSF + 500 μL buffer + 25 μL 100 μg/mL 3-methylcytidine methosulfate in water, mix, add to the SPE column, wash with five 1 mL portions of buffer, elute with two 1 mL portions of 100 mM formic acid. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject the whole amount. Buffer was 250 mM pH 8.8 ammonium acetate.)

HPLC VARIABLES
Column: two 300 × 4 μBondapak C18 columns in series
Mobile phase: 10 mM Ammonium phosphate adjusted to pH 2.5 with 85% phosphoric acid
Flow rate: 1.5
Injection volume: 100
Detector: UV 235

CHROMATOGRAM
Retention time: 8.0
Internal standard: 3-methylcytidine methosulfate (11.0)
Limit of detection: 100 nM

OTHER SUBSTANCES
Noninterfering: acetaminophen, N-acetylprocainamide, acyclovir, amikacin, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, amitriptyline, azidothymidine, caffeine, carbamazepine, chloramphenicol, cyclosporin A , cytidine, desipramine, diazepam, digoxin, disopyramide, ethosuximide, gentamicin, imipramine, kanamycin, lidocaine, lithium, methotrexate, 1-methyladenosine, 5-methylcytidine, 7-methylguanosine, 7-methylinosine, netilmicin, nortriptyline, phenobarbital, phenytoin, primidone, procainamide, quinidine, salicylic acid, theophylline, 2-thiocytidine, tobramycin, uridine, valproic acid, vancomycin, zalcitabine

KEY WORDS
serum; plasma; SPE

REFERENCE
Granich,G.G.; Krogstad,D.J.; Connor,J.D.; Desrochers,K.L.; Sherwood,C. High-performance liquid chromatography (HPLC) assay for ribavirin and comparison of the HPLC assay with radioimmunoassay, *Antimicrob.Agents Chemother.*, **1989**, *33*, 311–315.

SAMPLE
Matrix: blood, tissue, tracheal aspirate
Sample preparation: Dilute tracheal aspirates 10-100 fold with 250 mM pH 8.8 ammonium acetate buffer. Homogenize (glass/PTFE homogenizer) mouse lung with 1 mL water, centrifuge at 13000 g. Filter (Amicon CF25) 1 mL plasma, serum, diluted tracheal aspirate, or lung homogenate while centrifuging at 500 relative centrifugal force (RCF) for 30 min, dilute ultrafiltrate with a volume of 2.5 M pH 8.8 ammonium acetate buffer equal to 10% of the volume of ultrafiltrate, add the mixture to the SPE cartridge, wash with 7 mL 250 mM pH 8.8 ammonium acetate buffer, elute with 6 mL 100 mM formic acid. Lyophilize the eluate, reconstitute with a volume of mobile phase equal to the original volume of ultrafiltrate, inject a 50 μL aliquot. (Soak the CF25 filters in water for 1 h before use. Prepare SPE cartridges by slurrying Matrex PBA-60 gel (88.87 μmole boron per mL, Amicon) in 250 mM pH 8.8 ammonium acetate and packing it into a 40 × 7 column to a bed volume of 1 mL, pass 50 mL 250 mM pH 8.8 ammonium acetate through the column.)

HPLC VARIABLES
Guard column: Guard Pak C18 (Waters)
Column: 250 × 4.6 5 μm Microsorb C18
Mobile phase: MeOH:buffer 1:99 adjusted to pH 5.10 with 10% ammonium hydroxide or 8% phosphoric acid (Buffer was 20 mM ammonium phosphate. Following each analysis flush column with a gradient of MeCN:water from 0:100 to 80:20.)
Flow rate: 1
Injection volume: 50
Detector: UV 207

CHROMATOGRAM
Retention time: 5.3
Limit of detection: 100 ng/mL

KEY WORDS
mouse; lung; ultrafiltrate; SPE

REFERENCE
Smith,R.H.A.; Gilbert,B.E. Quantification of ribavirin in biological fluids and tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *414*, 202–210.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. 1 mL Serum + 5 μg uridine, vortex, add 1 mL water, vortex, filter (Centricon 30 membrane, 30 000 exclusion limit) while centrifuging at 2750 g for 2 h, wash the filtrate with 1.5 mL dichloromethane, pass the aqueous layer through a 10 mm i.d. column containing 2.3 mL wet 50-100 mesh Dowex 1X4-100 (chloride form) (Sigma) on top of 0.8 mL wet 60-150 mesh Lewatit S-1080 (hydrogen form) (Merck), elute with 3 mL water. Collect all the eluate and evaporate it to dryness under vacuum at 30°. reconstitute in 600 μL water, inject a 20-100 μL aliquot. Urine. 200 μL Urine + 5 μg uridine, vortex for a few s, add the urine mixture dropwise with stirring to 3 mL MeOH, centrifuge at 10° at 10000 g for 20 min. Remove the supernatant and add it to 2 mL n-hexane, mix, discard the hexane layer. Evaporate the MeOH layer to dryness under a nitrogen and vacuum, reconstitute the residue in 3 mL water, inject a 100 μL aliquot (J. Chromatogr. 1978, 160 169).

HPLC VARIABLES
Guard column: Guard Pak RP-18 (Waters)
Column: 250 × 5 7 μm LiChrosorb C18
Mobile phase: Water
Flow rate: 1
Injection volume: 20-100
Detector: UV 207

CHROMATOGRAM
Retention time: 6.9
Internal standard: uridine (13)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; ultrafiltrate

REFERENCE
Paroni,R.; Sirtori,C.R.; Borghi,C.; Kienle,M.G. High-performance liquid chromatographic determination of ribavirin in serum and urine and of its urinary metabolite 1,2,4-triazole-3-carboxamide, *J.Chromatogr.*, **1987**, *420*, 189–196.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize hamster brain with phosphate-buffered saline, centrifuge at 1600 g for 10 min. Remove the supernatant and add it to EtOH, heat at 90° to remove EtOH, suspend the protein-free sample in mobile phase, centrifuge at 8300 g for 5 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: TSKgel ODS-120T (Tosoh)
Mobile phase: MeCN:25 mM pH 2.5 buffer 2:98
Flow rate: 1
Injection volume: 10
Detector: UV 226

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 10 μg/g

KEY WORDS
hamster; brain; some interference from endogenous substances; pharmacokinetics

REFERENCE
Ishii,T.; Hosoya,M.; Mori,S.; Shigeta,S.; Suzuki,H. Effective ribavirin concentration in hamster brains for antiviral chemotherapy for subacute sclerosing panencephalitis, *Antimicrob.Agents Chemother.*, **1996**, *40*, 241–243.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine ten-fold with water, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

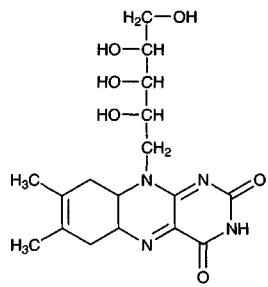
HPLC VARIABLES
Column: 250 × 4.6 Lichrosorb RP18
Mobile phase: Water
Flow rate: 1
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 5 μg/mL

KEY WORDS
mouse

REFERENCE
Ryan,D.M.; Ticehurst,J.; Dempsey,M.H.; Penn,C.R. Inhibition of influenza virus replication in mice by GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetylneuramic acid) is consistent with extracellular activity of viral neuraminidase (sialidase), *Antimicrob.Agents Chemother.*, **1994**, *38*, 2270–2275.

Riboflavin



Molecular formula: $C_{17}H_{20}N_4O_6$
Molecular weight: 376.37
CAS Registry No.: 83-88-5, 130-40-5 (phosphate sodium)
Merck Index: 8367

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon 25000 molecular-weight cut-off) serum, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 25 μm pellicular reversed-phase (Whatman)
Column: 300 × 3.9 μBondapak
Mobile phase: Gradient. A was 20 mM pH 5.6 KH_2PO_4. B was MeOH:water 60:40. A:B from 100:0 to 60:40 over 35 min.
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: creatinine, cytidine, hypoxanthine, inosine, kynurenine, 5-methylcytidine, tryptophan, tyrosine, uric acid, xanthine

KEY WORDS
dog; human; serum

REFERENCE
Assenza,S.P.; Brown,P.R. Comparison of high-performance liquid chromatographic serum profiles of humans and dogs, *J.Chromatogr.*, **1980**, *181*, 169–176.

SAMPLE
Matrix: blood, formulations, urine
Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma, urine. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. Mix 40 μL plasma or 100 μL urine with twice the volume of MeCN for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-18
Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min
Flow rate: 1
Injection volume: 10
Detector: UV 270

CHROMATOGRAM
Retention time: 19.67
Internal standard: xanthine (4.65)
Limit of detection: 2.5 ng

OTHER SUBSTANCES
Extracted: ascorbic acid, folic acid, niacin, niacinamide, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 267.7

CHROMATOGRAM
Retention time: 7.182

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 17
Limit of quantitation: ≤50 ng/mL

OTHER SUBSTANCES
Extracted: thiamine, pyridoxine, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albalá-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: pantothenic acid, thiamine, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: niacinamide, thiamine, pyridoxine, ascorbic acid (UV 254)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil

Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4 (step gradient), 8 (curve gradient), 2 (or riboflavin-5'-phosphate (either system))

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacin, niacinamide, pyridoxamine (UV 280), thiamine, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, pyridoxine, thiamine, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5

Detector: UV 220

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, saccharin, thiamine, pyridoxine, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 100 × 2.1 3 μm Spherisorb ODS-2
Mobile phase: MeOH:buffer 20:80 containing 0.1% triethylamine (Buffer was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid.)
Flow rate: 0.2 for 5 min, to 0.3 over 0.5 min, 0.3 for 12.5 min
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 16
Limit of detection: 1.80 ng

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), pyridoxine (UV 280), niacin, niacinamide, thiamine

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light; narrow bore

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1
Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 11.12
Internal standard: theobromine (8)
Limit of detection: 0.465 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, niacinamide, thiamine, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10 :90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, thiamine, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, pyridoxine, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4

Detector: UV 254

CHROMATOGRAM
Retention time: 4.2

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, thiamine, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:50 mM KH_2PO_4 90:10
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 10.5

OTHER SUBSTANCES
Simultaneous: biotin, folic acid, niacin, pantothenic acid, niacinamide

REFERENCE
MetaChem Catalog, **1995,** p. 21.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Nucleosil C18 SPE cartridge with 2 mL MeOH, 2 mL MeOH containing 5 mM sodium heptanesulfonate, and two 2 mL portions of water. Suspend 5 g homogenized tissue with 35 mL 10 mM HCl, autoclave at 121° for 30 min, add 2 mL 25 mg/mL taka-distase (Fluka) in 2.5 M sodium acetate, add 2 mL 10 (muscle) or 20 (liver) mg/mL clara-distase (Fluka) in water, add 2 mL 50 mg/mL papain (Merck) in water, adjust pH to 4.5, heat at 37° for 16-18 h, filter (paper), adjust pH to 6.5, filter again, make up to 50 mL with water, add 4 mL to the SPE cartridge, wash with 2 mL MeOH:water 20:80 containing 5 mM sodium heptanesulfonate, elute with 2 mL MeOH:water 50:50 containing 5 mM sodium heptanesulfonate, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 μm Nucleosil C18
Column: 150 × 4.6 3 μm Nucleosil C18
Mobile phase: MeCN:10 mM pH 3.0 KH_2PO_4 16:84 (muscle) or 15:85 (liver) containing 5 mM sodium heptane sulfonate (Wash with MeCN:water 20:80 at the end of the day, store column in MeCN.)
Column temperature: 45
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5 (muscle), 5 (liver)
Limit of detection: 160 ng/g

OTHER SUBSTANCES
Extracted: thiamine

KEY WORDS
pig; muscle; liver; protect from light; SPE

REFERENCE

Barna,I.; Dworschák,E. Determination of thiamine (vitamin B1) and riboflavin (vitamin B2) in meat and liver by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 359–363.

SAMPLE

Matrix: urine
Sample preparation: Centrifuge urine at 1400 g for 10 min, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:buffer 35:65 (Buffer was 10 mM KH_2PO_4 adjusted to pH 5.0 with 1 M NaOH.)
Flow rate: 2
Injection volume: 50
Detector: F ex 320-400 em 400-700 (filter)

CHROMATOGRAM

Retention time: 4.5 (riboflavin), 3 (riboflavin-5-phosphate)
Limit of detection: 50 ng/mL

KEY WORDS

protect from light

REFERENCE

Smith,M.D. Rapid method for determination of riboflavin in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 285–291.

SAMPLE

Matrix: urine
Sample preparation: Preserve urine with oxalic acid, centrifuge at 2000 g for 10 min, inject a 25-100 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:water 34:66
Flow rate: 1
Injection volume: 25-100
Detector: F ex 450 em 530 (long-pass filter)

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: lumichrome, lumiflavin
Noninterfering: acriflavine, apresoline, corticosterone, dipyridamole, estradiol, estriol, estrone, ethylenediamine, hydrocortisone, prednisolone, succinic acid, tetrahydrocortisone, testosterone, urobilin

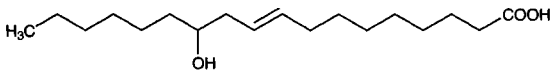
KEY WORDS

protect from light

REFERENCE

Gatautis,V.J.; Naito,H.K. Liquid-chromatographic determination of urinary riboflavin, *Clin.Chem.*, **1981**, *27*, 1672–1675.

Ricinoleic acid



Molecular formula: $C_{18}H_{34}O_3$
Molecular weight: 298.47
CAS Registry No.: 141-22-0
Merck Index: 8378

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.17-1.7 mg/mL solution in MeOH, inject a 30 μL aliquot.

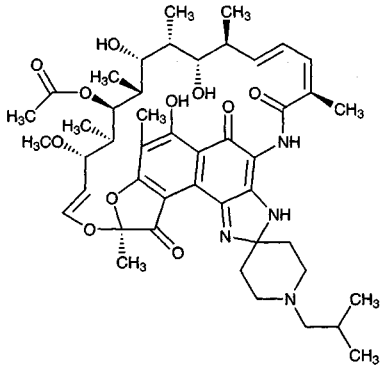
HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH containing 0.05% acetic acid. B was water containing 0.05% acetic acid. A:B 85:15 to 100:0 over 40 min, maintain at 100:0.
Flow rate: 1
Injection volume: 30
Detector: evaporative light scattering (ELSD, MK VIII, Varex), drift tube 75°, nitrogen flow 1 L/min, nitrogen pressure 22 psi or UV 205

CHROMATOGRAM
Retention time: 8.95

OTHER SUBSTANCES
Simultaneous: linoleic acid, fatty acids

REFERENCE
Lin,J.-T.; McKeon,T.A.; Stafford,A.E. Gradient reversed-phase high-performance liquid chromatography of saturated, unsaturated and oxygenated free fatty acids and their methyl esters, *J.Chromatogr.A*, **1995**, *699*, 85–91.

Rifabutin



Molecular formula: $C_{46}H_{62}N_4O_{11}$
Molecular weight: 847.02
CAS Registry No.: 72559-06-9
Merck Index: 8380

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C8 SPE cartridge with 1 mL MeOH and 1 mL water. 1 mL Plasma + 25 μL 2 μg/mL sulindac in water, vortex briefly, centrifuge at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 RP-8 (Brownlee, ABI)

Column: 250 × 4.6 5 μm Zorbax RX C8
Mobile phase: MeCN:buffer 47:53 (Buffer was 50 mM KH_2PO_4 containing 50 mM sodium acetate, pH adjusted to 4.0 with acetic acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM
Retention time: 10.8
Internal standard: sulindac (6.9)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
plasma; protect from light; pharmacokinetics; rugged; SPE

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifabutin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 125–130.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 500 μL 50 mM pH 7.4 KH_2PO_4, mix, add 2 mL dichloromethane:isooctane 40:60, vortex for 10 min, centrifuge at 1200 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 300 μL mobile phase, add 1 mL n-hexane, wash, centrifuge at 1200 g for 5 min, discard hexane layer, repeat wash, inject a 200 μL aliquot of the aqueous phase. Urine. Inject an aliquot directly.

HPLC VARIABLES
Guard column: 30-38 μm CO:PELL ODS
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM KH_2PO_4 40:60
Flow rate: 1
Injection volume: 200
Detector: UV 275

CHROMATOGRAM
Retention time: 24.0
Limit of detection: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat; human; rabbit; monkey

REFERENCE
Battaglia,R.; Pianezzola,E.; Salgarollo,G.; Zini,G.; Strolin Benedetti,M. Absorption, disposition and preliminary metabolic pathway of ^{14}C-rifabutin in animals and man, *J.Antimicrob.Chemother.*, **1990**, *26*, 813–822.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 20 μL 5 μg/mL medazepam in MeOH + 1 mL buffer + 7 mL hexane:ethyl acetate 80:20, vortex for 10 s, centrifuge at 1200 rpm for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at 40°, reconstitute with 250 μL hexane:ethyl acetate 80:20, add 200 μL 50 mM phosphoric acid, vortex for 10 s, centrifuge at 1200 rpm for 1-2 min, freeze in dry ice/isopro-

panol, discard the organic layer, wash the frozen aqueous layer with 1 mL hexane, thaw, place under reduced pressure at room temperature for 10 min, add 100 μL 250 mM ammonium acetate in MeOH, mix, inject a 100 μL aliquot. Urine. 1 mL Urine + 20 μL 50 μg/mL medazepam in MeOH + 50 μL 3.6 M aqueous sulfuric acid, mix. Remove a 200 μL aliquot and add it to 800 μL 100 mM ammonium acetate in MeOH:water 40:60, mix, inject a 100 μL aliquot. (Buffer was 250 mM KH_2PO_4 containing 50 mM sodium 1-heptanesulfonate adjusted to pH 7.4.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 38:62 (Buffer was 50 mM KH_2PO_4:triethylamine 61.5:0.5 adjusted to pH 4.2 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 275

CHROMATOGRAM
Retention time: 22
Internal standard: medazepam (19)
Limit of quantitation: 100 ng/mL (urine), 5 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: clofazimine
Noninterfering: amikacin, isoniazid, streptomycin, zidovudine

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KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lewis,R.C.; Hatfield,N.Z.; Narang,P.K. A sensitive method for quantitation of rifabutin and its desacetyl metabolite in human biological fluids by high-performance liquid chromatography (HPLC), *Pharm.Res.*, **1991**, *8*, 1434–1440.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 208.7

CHROMATOGRAM
Retention time: 17.583

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations, tissue
Sample preparation: Tissue. Homogenize liver slices with 200 μL water and about 250 mg 1 mm glass beads, using a Mini Bead Beater (Biospec Products), extract with 1 mL MeCN. Extract a 250 μL aliquot of the slice incubation medium with 1 mL MeCN. Mix, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot. Microsomal incubations. Add 1 mL MeCN to 1 mL microsomal incubation, mix, centrifuge at 1900 g for 15 min at 4°. Extract a 200 μL aliquot of the supernatant with 1 mL MeCN, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18 Sentry Guard (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:10 mM pH 4.0 ammonium phosphate triethylamine50:49.9:0.1
Flow rate: 1
Detector: UV 278

CHROMATOGRAM
Retention time: 31.6

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Jamis-Dow,C.A.; Katki,A.G.; Collins,J.M.; Klecker,R.W. Rifampin and rifabutin and their metabolism by human liver esterases, *Xenobiotica*, **1997**, *27*, 1015–1024.

SAMPLE
Matrix: tissue
Sample preparation: Vortex 200 μL incubation mixture and 1 mL MeOH containing 0.1% acetic acid, immerse in ice for 15 min, centrifuge at 6500 g for 5 min, evaporate the supernatant to dryness under nitrogen, reconstitute the residue in 50 μL mobile phase, combine two aliquots, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil C18
Mobile phase: Gradient. A was 3% triethylamine containing 0.3% trifluoroacetic acid adjusted to pH 2 with phosphoric acid. B was MeCN. A:B from 73:27 to 58:42 over 45 min
Flow rate: 1.5
Injection volume: 75
Detector: Radioactivity, Radiomatic A-500 (Flow Scintillation Analyzer)

CHROMATOGRAM
Retention time: 35

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver; enterocytes; intestine

REFERENCE
Iatsimirskaia,E.; Tulebaev,S.; Storozhuk,E.; Utkin,I.; Smith,D.; Gerber,N.; Koudriakova,T. Metabolism of rifabutin in human enterocyte and liver microsomes: kinetic parameters, identification of enzyme systems, and drug interactions with macrolides and antifungal agents, *Clin.Pharmacol.Ther.*, **1997**, *61*, 554–562.

SAMPLE
Matrix: urine
Sample preparation: Inject a 50-200 μL aliquot of urine directly.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. MeCN:50 mM pH 4.5 KH_2PO_4 40:60 for 10 min, to 60:40 over 5 min, maintain at 60:40 for 5 min.
Flow rate: 1 for 10 min then 1.5
Injection volume: 50-200
Detector: UV 275 or radioactivity

CHROMATOGRAM
Retention time: 16.5

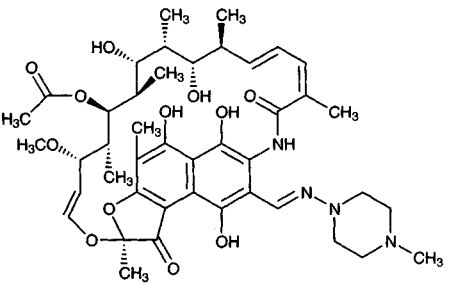
OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat

REFERENCE
Battaglia,R.; Salgarollo,G.; Zini,G.; Montesanti,L.; Strolin Benedetti,M. Absorption, disposition, and urinary metabolism of ^{14}C-rifabutin in rats, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1391–1396.

Rifampin

Molecular formula: $C_{43}H_{58}N_4O_{12}$
Molecular weight: 822.95
CAS Registry No.: 13292-46-1
Merck Index: 8382



SAMPLE
Matrix: blood
Sample preparation: Add 400 μL 1 M pH 4.0 phosphate buffer and 40 μL 10 mg/mL ascorbic acid solution to 1 mL plasma. Mix for 10 s, add 4 mL ethyl acetate. Vortex for 5 min, centrifuge at 1800 g for 10 min. Remove a 3 mL aliquot of the aqueous layer and evaporate it to dryness under nitrogen at 100°. Reconstitute the residue with 500 μL MeOH, vortex for 1 min. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb
Mobile phase: MeOH:10 mM pH 5.5 phosphate buffer 68:32
Column temperature: 28
Flow rate: 1

Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 4.33
Internal standard: rifampin

OTHER SUBSTANCES
Extracted: rifapentine

KEY WORDS
serum; rifampin is IS

REFERENCE
He,X.; Wang,J.; Liu,X.; Chen,X. High-performance liquid chromatography assay of rifapentine in human serum, *J.Chromatogr.B*, **1996**, *681*, 412–415.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL MeCN to 100 μL plasma, vortex for 10 s, centrifuge for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C8
Mobile phase: MeCN:MeOH:10 mM potassium dihydrogen phosphate 35:5:60
Flow rate: 1
Injection volume: 20
Detector: UV 334

CHROMATOGRAM
Retention time: 6.5
Limit of quantitation: 200 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Le Guellec,C.; Gaudet,M.-L.; Lamanetre,S.; Breteau,M. Stability of rifampin in plasma: Consequences for therapeutic monitoring and pharmacokinetic studies, *Ther.Drug Monit.*, **1997**, *19*, 669–674.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL 100 mM HCl just before use. 500 μL Plasma + 500 μL 100 mM HCl + 50 μL 19 μg/mL sulindac in MeOH, vortex briefly, add to SPE cartridge, wash with 1 mL 100 mM HCl, elute with 400 μL MeOH:MeCN 60:40, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18 RCSS Guard Pak
Column: 100 × 8 4 μm Nova Pak C18 Radial Pak
Mobile phase: MeCN:buffer 42:58 (Buffer was 50 mM sodium citrate adjusted to pH 4.3 with 50 mM HCl.)
Flow rate: 2.3
Injection volume: 20
Detector: UV 342

CHROMATOGRAM
Retention time: 4.65
Internal standard: sulindac (3.07)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Swart,K.J.; Papgis,M. Automated high-performance liquid chromatographic method for the determination of rifampicin in plasma, *J.Chromatogr.*, **1992**, *593*, 21–24.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 50 μL 50 μg/mL N-butarylaminophenol + 400 μL 10% acetic acid + 7 mL diethyl ether:dichloromethane 2:1, shake, centrifuge at 2059 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 30 μm C8
Column: 250 × 4.6 5 μm Spherisorb C8
Mobile phase: Gradient. MeCN:5 mM pH 3.5 phosphate buffer from 6:94 to 90:10 over 5 min, maintain at 90:10 for 12 min.
Flow rate: 2
Injection volume: 20
Detector: UV 248

CHROMATOGRAM
Retention time: 11.9
Internal standard: N-butarylaminophenol (4.22)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: pyrazinamide

KEY WORDS
plasma

REFERENCE
Walubo,A.; Smith,P.; Folb,P.I. Comprehensive assay for pyrazinamide, rifampicin and isoniazid with its hydrazine metabolites in human plasma by column liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 391–396.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 15 mg SPEC.C8 SPE disc (ANSYS) or a Bond-Elut C8 SPE cartridge with two 1 mL portions of MeCN and two 1 mL portions of 100 mM HCl. 500 μL Serum + 200 μL 5% ascorbic acid in 100 mM HCl + 100 μL 50 μg/mL papaverine hydrochloride in water, mix well, add to the SPE disc or cartridge, apply a vacuum for 20 min, elute with five 100 μL portions of MeCN:MeOH 60:40. Evaporate the eluate to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 300 μL 100 mM HCl:water 67:33, inject a 50 μL aliquot. (Disc gave higher recoveries than cartridge.)

HPLC VARIABLES
Guard column: ODS
Column: 250 × 4.6 Spherisorb ODS-1
Mobile phase: MeCN:100 mM pH 4.7 KH_2PO_4 35:65
Column temperature: 40
Flow rate: 1.2
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 11.5
Internal standard: papaverine (14.9)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE

REFERENCE
Ye,L.; Stewart,J.T.; Zhang,H. A comparison of disc and cartridge solid-phase extraction for the LC determination of rifampin and 25-desacetylrifampin in human serum, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1185–1188.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C2 SPE cartridge with 1 mL MeOH and 1 mL water. 500 µL Plasma + 25 µL 2 µg/mL sulindac in water + 100 µL 100 mM HCl, vortex briefly, centrifuge at 630 rpm for 5 min, add to the SPE cartridge, wash with 1 mL water, elute with 1 mL MeOH, add the eluate to 500 µL 3 mg/mL ascorbic acid, inject a 150 µL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 µm RP-8 (Brownlee, ABI)
Column: 250 × 4.6 5 µm Zorbax RX C8
Mobile phase: MeCN:50 mM KH_2PO_4 45:55
Flow rate: 1
Injection volume: 150
Detector: UV 340

CHROMATOGRAM
Retention time: 4.4
Internal standard: sulindac (7.8)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: atevirdine, delavirdine, U-89,255, U-96,183

KEY WORDS
plasma; protect from light; pharmacokinetics; rugged; SPE

REFERENCE
Lau,Y.Y.; Hanson,G.D.; Carel,B.J. Determination of rifampin in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, **1996**, *676*, 147–152.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 µL CSF or plasma + dimethylaminobenzoic acid + 200 µL MeOH, mix, add 1 mL buffer, add 7 mL dichloromethane:diethyl ether 40:60, shake for 15 min, centrifuge at 2500 g for 10 min. Remove the organic layer and evaporate it to dryness AT 45°, reconstitute the residue in 50 µl MeOH, vortex for 10 s, inject a 25 µL aliquot. (Buffer was 1 M KH_2PO_4 containing 0.2% ascorbic acid, pH adjusted to 4.2 with 1 M HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 30 µm C8
Column: 250 × 4.6 5 µm Lichrosorb RP-8
Mobile phase: MeCN:10 mM pH 3.5 phosphate buffer 48:52
Flow rate: 1.5
Injection volume: 25
Detector: UV 215

CHROMATOGRAM
Retention time: 5.8
Internal standard: dimethylaminobenzoic acid (3.8)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: p-aminosalicylic acid, pyrazinamide, isoniazid

KEY WORDS
plasma; rabbit

REFERENCE
Chan,K. Rifampicin concentrations in cerebrospinal fluid and plasma of the rabbit by high performance liquid chromatography, *Methods Find.Exp.Clin.Pharmacol.*, **1986**, *8*, 721–726.

SAMPLE
Matrix: blood, CSF
Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM
Retention time: 12.93, 16.28, 17.16 (compound undergoes decomposition)
Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES
Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, trimeprazine, trimethoprim

KEY WORDS
plasma; serum; column-switching

REFERENCE
Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE
Matrix: blood, saliva, urine
Sample preparation: 1 mL Plasma, urine, or saliva + 50 μL MeOH + 1 mL buffer + 1 mL isooctane:dichloromethane 60:40, shake mechanically at 350 rpm for 10 min, centrifuge at 2000 g for 5 min, inject a 5-200 μL aliquot of the supernatant. (Buffer was 2 g ascorbic acid and 10 g anhydrous sodium sulfate made up to 50 mL with concentrated pH 6 buffer solution (Merck Cat. No. 9886), stir continuously, prepare fresh each day.)

HPLC VARIABLES
Column: 100 × 7.5 5 μm LiChrosorb Si 60
Mobile phase: Isooctane:dichloromethane:EtOH:water:acetic acid 45:36.6:16.8:1.65:0.002

Flow rate: 3
Injection volume: 5-200
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Limit of detection: 20 ng/mL (Clin.Pharmacol.Ther. 1991, 50, 682)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lecaillon,J.B.; Febvre,N.; Metayer,J.P.; Souppart,C. Quantitative assay of rifampicin and three of its metabolites in human plasma, urine and saliva by high-performance liquid chromatography, *J.Chromatogr.*, **1978**, *145*, 319–324.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 236.9

CHROMATOGRAM
Retention time: 16.167

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of

maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 225.2

CHROMATOGRAM
Retention time: 20.85

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min, remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 4.7 KH_2PO_4 40:60
Flow rate: 1
Injection volume: 75
Detector: UV 340

CHROMATOGRAM
Retention time: 6.2
Internal standard: papaverine
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 208TP54 C8
Mobile phase: MeCN:buffer 32.8:67.2 (Buffer was 50 mM K_2HPO_4 adjusted to pH 6.5 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 6.8

OTHER SUBSTANCES
Simultaneous: minocycline, degradation products

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Pearson,S.D.; Trissel,L.A. Stability and compatibility of minocycline hydrochloride and rifampin in intravenous solutions at various temperatures, *Am.J.Hosp.Pharm.*, **1993**, *50*, 698–702.

SAMPLE
Matrix: microsomal incubations, tissue
Sample preparation: Tissue. Homogenize liver slices with 200 μL water and about 250 mg 1 mm glass beads, using a Mini Bead Beater (Biospec Products), extract with 1 mL MeCN. Extract a 250 μL aliquot of the slice incubation medium with 1 mL MeCN. Mix, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot. Microsomal incubations. Add 1 mL MeCN to 1 mL microsomal incubation, mix, centrifuge at 1900 g for 15 min at 4°. Extract a 200 μL aliquot of the supernatant with 1 mL MeCN, centrifuge at 11000 g for 4 min at 4°. Dry the supernatant under vacuum, reconstitute the residue with 150 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: Symmetry C18 Sentry Guard (Waters)
Column: 250 × 4.6 5 μm Symmetry C18 (Waters)
Mobile phase: MeCN:10 mM pH 4.0 ammonium phosphate:triethylamine 30:69.9:0.1
Flow rate: 1
Detector: UV 240

CHROMATOGRAM
Retention time: 34.4

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
Jamis-Dow,C.A.; Katki,A.G.; Collins,J.M.; Klecker,R.W. Rifampin and rifabutin and their metabolism by human liver esterases, *Xenobiotica*, **1997**, *27*, 1015–1024.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge and filter cell solutions (0.22 μm), inject an aliquot.

HPLC VARIABLES
Guard column: Guard-PAK C18 (Waters)
Column: 150 × 3.9 5 μm NOVA PAK C18
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 40:60
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 3.3

REFERENCE
Koga,H. High-performance liquid chromatography measurement of antimicrobial concentrations in polymorphonuclear leukocytes, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1904–1908.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 5 mg/mL solution in MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4.6 Hypersil C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: MS, HP 5985B quadrupole MS, direct liquid introduction interface, 5 μm orifice, water-cooled stainless steel diaphragm, ion source pressure 1 Torr, ion source 250°, LC eluent used as reagent gas

OTHER SUBSTANCES
Also analyzed: rifapentine, rifamycin SV

REFERENCE
Vékey,K.; Edwards,D.M.F.; Zerilli,L.F. Liquid chromatographic-mass spectrometric studies on rifamycin antibiotics, *J.Chromatogr.*, **1989**, *474*, 317–327.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Excalibar C18-CN (Alltech)
Mobile phase: MeOH:5 mM tetra-n-butylammonium hydroxide 80:20 adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 4.3
Internal standard: rifampin

OTHER SUBSTANCES
Simultaneous: isoniazid, pyrazinamide

KEY WORDS
rifampin is IS

REFERENCE
Gaitonde,C.D.; Pathak,P.V. Rapid liquid chromatographic method for the estimation of isoniazid and pyrazinamide in plasma and urine, *J.Chromatogr.*, **1990**, *532*, 418–423.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 50 μg/mL solution in MeOH:water 50:50 (electrospray) or a 400 μg/mL solution in MeOH:200 mM ammonium acetate 50:50 (thermospray), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:200 mM ammonium acetate 60:40
Flow rate: 1.2
Injection volume: 20
Detector: MS, Delsi/Nermag R3010 triple quadrupole, Delsi/Nermag electrospray interface, flow rate 1 μL/min into source or Finnigan MAT TSQ 70 triple quadrupole, Finnigan TSPI interface and source, positive ion, discharge off, filament off, vaporizer 75°, block (jet) 330

CHROMATOGRAM
Retention time: 13.5

OTHER SUBSTANCES
Simultaneous: rifamycin SV, rifamycin B

KEY WORDS
electrospray; thermospray

REFERENCE
Korfmacher,W.A.; Bloom,J.; Churchwell,M.I.; Getek,T.A.; Hansen,E.B.,Jr.; Holder,C.L.; McManus,K.T. Characterization of three rifamycins via electrospray mass spectrometry and HPLC-thermospray mass spectrometry, *J.Chromatogr.Sci.*, **1993**, *31*, 498–501.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 160 × 4 5 μm Zorbax Rx-C8
Mobile phase: MeOH:buffer 70:30 (Buffer was 5 mM tetrabutylammonium hydroxide adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.7
Injection volume: 10
Detector: UV 265

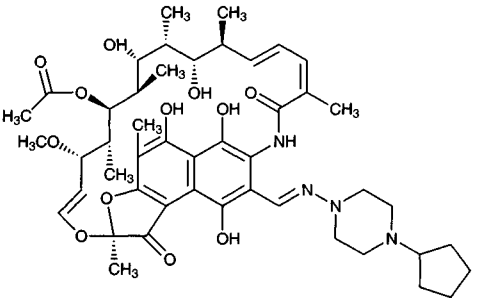
CHROMATOGRAM
Retention time: 3.8

OTHER SUBSTANCES
Simultaneous: pyrazinamide

REFERENCE
Nahata,M.C.; Morosco,R.S.; Peritore,S.P. Stability of pyrazinamide in two suspensions, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1558–1560.

Rifapentine

Molecular formula: $C_{47}H_{64}N_4O_{12}$
Molecular weight: 877.04
CAS Registry No.: 61379-65-5
Merck Index: 8385



SAMPLE
Matrix: blood
Sample preparation: Add 400 μL 1 M pH 4.0 phosphate buffer, 40 μL 10 mg/mL ascorbic acid solution, and 50 μL 100 μg/mL rifampin solution to 1 mL plasma. Mix for 10 s, add 4 mL ethyl acetate. Vortex for 5 min, centrifuge at 1800 g for 10 min. Remove a 3 mL aliquot of the aqueous layer and evaporate it to dryness under nitrogen at 100°. Reconstitute the residue with 500 μL MeOH, vortex for 1 min. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C18

Mobile phase: MeOH:10 mM pH 5.5 phosphate buffer 68:32
Column temperature: 28
Flow rate: 1
Injection volume: 20
Detector: UV 336

CHROMATOGRAM
Retention time: 6.83
Internal standard: rifampin (4.33)
Limit of quantitation: 5 ng

KEY WORDS
serum

REFERENCE
He,X.; Wang,J.; Liu,X.; Chen,X. High-performance liquid chromatography assay of rifapentine in human serum, *J.Chromatogr.B*, **1996**, *681*, 412–415.

SAMPLE
Matrix: blood
Sample preparation: Filter (0.45 μm) plasma, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelcosil LC HISEP
Column: 150 × 4.6 Supelcosil LC HISEP (Invert column between runs to prevent clogging.)
Mobile phase: Gradient. A was MeCN:2.6 mM 2-(N-morpholino)ethanesulfonic acid 5:95 containing 1% trifluoroacetic acid, adjusted to pH 6.5 with ethanolamine. B was MeCN:THF:2.6 mM 2-(N-morpholino)ethanesulfonic acid 20:10:70 containing 1% trifluoroacetic acid, adjusted to pH 6.5 with ethanolamine. A:B 70:30 for 4 min, to 30:70 over 24 min, re-equilibrate for 6 min.
Flow rate: 1.4
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 21
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; direct injection

REFERENCE
Riva,E.; Merati,R.; Cavenaghi,L. High-performance liquid chromatographic determination of rifapentine and its metabolite in human plasma by direct injection into a shielded hydrophobic phase column, *J.Chromatogr.*, **1991**, *553*, 35–40.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 300 μL 10 μg/mL rifampin in 1% ascorbic acid solution, mix, keep at 4°, inject a 100 μL aliquot on column A with mobile phase A and elute to waste, after 6 min backflush the contents of column A onto column B with mobile phase B, after 4 min remove column A from the circuit, elute column B with mobile phase B and monitor the effluent from column B. Re-equilibrate column A with mobile phase A for 15 min.

HPLC VARIABLES
Column: A 40 × 20 37-50 μm Corasil RP C18; B 20 × 4.6 25-40 μm LiChrosorb RP-8 + 300 × 3.9 10 μm μBondapak C18

Mobile phase: A 50 mM pH 7.0 phosphate buffer; B MeCN:THF:50 mM pH 7.0 phosphate buffer 42:5:53
Flow rate: 1
Injection volume: 100
Detector: UV 332

CHROMATOGRAM
Retention time: 12.6
Internal standard: rifampin (7.2)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, p-aminosalicylic acid, aspirin, caffeine, cefuroxime, ciprofloxacin, ibuprofen, isonicotinic acid, theophylline, vitamin A, thiamine, riboflavin, pyridoxine, ascorbic acid

KEY WORDS
serum; column-switching; dog; pharmacokinetics

REFERENCE
Lee,H.S.; Shin,H.C.; Han,S.S.; Roh,J.K. High-performance liquid chromatographic determination of rifapentine in serum using column switching, *J.Chromatogr.*, **1992**, *574*, 175–178.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 5 mg/mL solution in MeOH, inject a 10 μL aliquot.

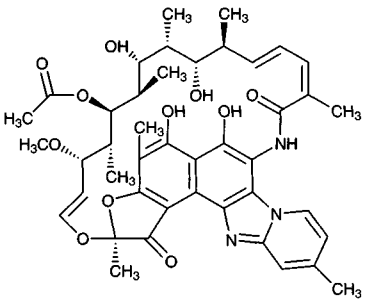
HPLC VARIABLES
Guard column: present but not specified
Column: 100 × 4.6 Hypersil C18
Mobile phase: MeOH
Flow rate: 1
Injection volume: 10
Detector: MS, HP 5985B quadrupole MS, direct liquid introduction interface, 5 μm orifice, water-cooled stainless steel diaphragm, ion source pressure 1 Torr, ion source 250°, LC eluent used as reagent gas

OTHER SUBSTANCES
Also analyzed: rifampin, rifamycin SV

REFERENCE
Vékey,K.; Edwards,D.M.F.; Zerilli,L.F. Liquid chromatographic-mass spectrometric studies on rifamycin antibiotics, *J.Chromatogr.*, **1989**, *474*, 317–327.

Rifaximin

Molecular formula: $C_{43}H_{51}N_3O_{11}$
Molecular weight: 785.89
CAS Registry No.: 80621-81-4
Merck Index: 8386



SAMPLE
Matrix: bile

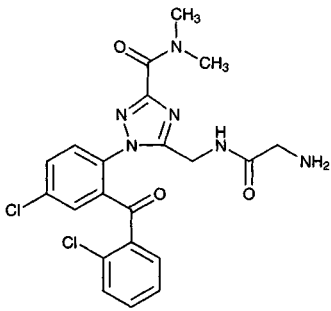
Sample preparation: 1 mL Bile + 4 mL pH 4.5 buffer + 200 µL 50 µg/mL IS in MeOH, extract twice with chloroform. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate to dryness, reconstitute the residue in 100 µL MeOH, inject an aliquot.

HPLC VARIABLES
Column: HSC18
Mobile phase: MeOH:1 mM pH 3 phosphate buffer 65:35
Column temperature: 35
Flow rate: 1
Detector: UV 276

CHROMATOGRAM
Internal standard: M 302

REFERENCE
Verardi,S.; Verardi,V. Bile rifaximin concentration after oral administration in patients undergoing cholecystectomy, *Farmaco*, **1990**, *45*, 131–135.

Rilmazafone



Molecular formula: $C_{21}H_{20}Cl_2N_6O_3$
Molecular weight: 475.33
CAS Registry No.: 99593-25-6
Merck Index: 8387

SAMPLE
Matrix: feed
Sample preparation: 1 g Feed + 10 mL MeOH, shake for 45 min, centrifuge at 2000 rpm for 10 min. Remove 5 mL of the supernatant and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 1 mL MeCN, add 100 µL 1 M tartaric acid containing 50 mM sodium 1-dodecanesulfonate, vortex for 10 min, add 5 mL n-hexane, vortex for 10 min, filter (0.45 µm) the aqueous layer, inject a 10 µL aliquot of the filtrate.

HPLC VARIABLES
Column: two 300 × 4 10 µm Nucleosil 10C18 columns in series
Mobile phase: MeCN:EtOH:buffer 35:12.5:52.5 (Buffer was 100 mM tartaric acid containing 5 mM sodium 1-dodecanesulfonate and 0.29 mM triethylamine.)
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 30

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Ikenishi,R.; Kitagawa,T. Analytical studies on 1-(2-o-chlorobenzoyl-4-chlorophenyl)-3-dimethylcarbamoyl-5-glycyl-aminomethyl-1H-1,2,4-triazole hydrochloride dihydrate. III. High-performance liquid chromatographic method applicable to animal feed, *Chem.Pharm.Bull.*, **1987**, *35*, 4544–4551.